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PRIMER NOTE

Microsatellite DNA markers for delineating population structure and kinship among the endangered Kirtland's warbler (*Dendroica kirtlandii*)

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Abstract

We document the isolation and characterization of 23 microsatellite DNA markers for the endangered Kirtland's warbler (*Dendroica kirtlandii*), a Nearctic/Neotropical migrant passerine. This suite of markers revealed moderate to high levels of allelic diversity (averaging 7.7 alleles per locus) and heterozygosity (averaging 72%). Genotypic frequencies at 22 of 23 (95%) markers conformed to Hardy–Weinberg equilibrium expectations, and no linkage disequilibrium was observed in blood samples taken from 14 warblers found on the wintering grounds in the Bahamas archipelago. Multilocus genotypes resulting from this suite of markers should reduce the amount of resources required for initiating new genetic studies assessing breeding structure, parentage, demographics, and individual-level ecological interactions for *D. kirtlandii*.

Keywords: Kirtland's warbler, microsatellite DNA, population structure, probability of identity

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Kirtland's warbler (*Dendroica kirtlandii*) is an endangered Nearctic/Neotropical migratory bird species, utilizing jack pine (*Pinus banksiana*) forests in Michigan, USA as summer nesting habitat and overwintering in the Bahamas archipelago. While recent demographic analyses suggest that individual warblers move regularly between geographical colonies (Probst *et al.* 2003), no data exist on population structuring or on levels of genetic diversity. Information on the extent of population structuring, extant levels of genetic diversity, and effective population size is essential to the evaluation of the current recovery program for *D. kirtlandii* and for any further consideration of the species' endangered status. To address these information needs, we have developed a suite of polymorphic microsatellite DNA markers for *D. kirtlandii*. Here we describe the isolation and characterization of 23 microsatellite markers and demonstrate the levels of diversity and heterozygosity among individuals collected on the overwintering grounds.

Blood was obtained by venipuncture of the brachial vein from 14 overwintering individuals on the island of Eleuthera, The Bahamas to develop microsatellite-enriched libraries. Blood was preserved on FTA cards (Whatman). Total genomic DNA was isolated from a portion of each blood-soaked FTA card using the Puregene DNA extraction kit (Gentra Systems) and resuspended in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). DNA concentrations were determined by fluorescence assay, and integrity of the DNA was visually inspected on 1% agarose gels.

Three microsatellite-enriched libraries were prepared for *D. kirtlandii* by Genetic Identification Services, Inc., Chatsworth, CA, USA (GIS; http://www.genetic-id-services.com/)

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Table 1 Characteristics of 23 Kirtland's warbler (*Dendroica kirtlandii*) microsatellite DNA loci: locus designation (GenBank Accession nos AY769671–AY769693), primer sequences, repeat motif, range of PCR product in (bp), number of alleles observed and average observed and expected heterozygosities. The observed level of heterozygosity at locus *Dki*D117 deviated from Hardy–Weinberg expectations (overall $\alpha = 0.05$, $P < 0.002$)

Locus	Primer sequences (5′–3′)	Repeat motif	Size (bp)	Alleles	H_O	H_E
*Dki*B3	F: GCTGTTGCTCCTACCATGAG	$(ATG)_{13}$	181–199	3	0.500	0.521
	R: AGGACTCAGTATTTGCGTCTGA					
*Dki*B12	F: ACATAAGGAGTGGAGCTCTTTG	$(ATG)_8$	181–208	7	0.857	0.746
	R: GCTGATGTTTGAAAGAGAGACC					
*Dki*B102	F: CAACAGGAGGACAAGGTAAGTC	$(ATG)_{10}$	248–275	6	0.769	0.800
	R: AGGACATCAGAACCATGTAACC					
*Dki*B106	F: CATCTGTGTCTTCTGCTGTGAG	$(ATG)_{15}$	171–195	6	0.692	0.717
	R: AACAGACAGTTAGTGACCTGGG					
*Dki*B108	F: ACTTCCCTTTTCTTCTCAAG	$(ATG)_{20}$	243–276	7	0.786	0.765
	R: GACAGCACTGGTTTTTCA					
*Dki*B118	F: AGCCGTCATTTCTCAGGC	$(ATG)_{14}$	200–212	5	0.636	0.792
	R: CAGCACCAGCAGCAAGTC					
*Dki*B119	F: ACAGGGACACTTCTCCTCTG	$(ATG)_{11}$	185–200	5	0.643	0.728
	R: TGCTTGATGGAATAGATTTCTG					
*Dki*B124	F: ATTCCAGAAGAAGGAGGAGAA	$(ATG)_7$	183–189	3	0.571	0.505
	R: GGATTTAGGGGATAACACCAG					
*Dki*C105	F: CTCTCCAGGCACAACAAG	$(TACA)_6$	169–181	4	0.923	0.668
	R: TCATGGTGGTTTTATCCTATG					
*Dki*C116	F: GTGCCTATTGAACAGTTAGACC	$(TACA)_{11}$	296–308	4	0.643	659
	R: AAGCCCCAAGTAGCTTTATAGT					
*Dki*C118	F: CTCTCCTTGCTCCCCTCTC	$(TACA)_8(TA)_2(TACA)_2$	301–309	3	0.214	0.204
	R: CACTTGCTGGCTGAACATC					
*Dki*D1	F: CTTTGGCAGTAATTCACACTTC	$(TAGA)_8(TGGA)_7$	261–297	10	0.929	0.886
	R: CTCTGGGGGAAGAATCTTTA					
*Dki*D10	F: GGTTGGTAGGTTGCTGAAC	$(GGTA)_{11}(TAGA)_{16}$	210–238	5	0.643	0.738
	R: AAAGCCTCAAAGTCTGTGC					
*Dki*D12	F: AAAAACTCAACTCTGGGTAAGG	$(TAGA)_{13}$	183–223	9	0.714	876
	R: GGCATCAAACACACAAGAAG					
*Dki*D102	F: GGGTCAGAGAGACACTGTTCT	$(TAGA)_{14}$	280–312	7	0.857	0.757
	R: CTCCCAGATTCAATCAGTTG					
*Dki*D104	F: AGGTTTACGGTTGAACTTGG	$(TAGG)_4(TAGA)_{15}$	192–252	10	0.929	0.910
	R: GCTCCTTTTCAGAAGCAAAG					
*Dki*D109	F: CTGGAGGTTAAGATTGTCTTC	$(TAGA)_{16}(CAGA)_2$	165–229	10	0.643	0.796
	R: GCAACTGTTTTAGGTAACTCA					
*Dki*D117	F: GCATTTTGAAGGAAGAATGTG	$(TAGA)_{19}(TAGG)_6$	270–272	8	0.500	0.854
	R: ACGTGGCTTATGCAAATACAT					
*Dki*D120	F: TGTATCACTTCTGATGCCACAC	$(TAGA)_{16}$	248–280	7	0.846	0.778
	R: CCTTAACCTTCTGCTTTCTCTG					
*Dki*D123	F: GGAGCCTCACAGTATTAGAAGG	$(TAGA)_{13}$	290–322	8	0.929	0.820
	R: AAGCAGTTTAATGCAAAGTGG					
*Dki*D124	F: TATGGCAAAAGAGAAAGTGC	$(TAGA)_{12}$	244–268	5	0.786	0.738
	R: GGAACAGCAGAAAATAAGGATA					
*Dki*D126	F: CCCCAGTGAACTCCTTTT	$(TAGA)_{16}$	167–203	9	0.786	0.812
	R: TCCCTTCTAAGCCAAACC					
*Dki*D128	F: CAATCCTTGTGTCATTATCCA	$(TAGA)_{14}$	255–311	8	0.769	0.840
	R: CCTGCTCTCTTTCAACATTATC					

using magnetic bead capture technology and the ATG, TACA, and TAGA microsatellite motif capture molecules (Peacock *et al.* 2002). Isolation and sequencing of plasmid DNA were performed as described in Eackles & King (2002). A total of 100 clones were screened for usable microsatellites. Of this group, 53 microsatellites were deemed unique, of sufficient length (> 10 repeats), and possessed adequate flanking regions for primer development. Primers were designed using the DESIGNER PCR program (version 1.03; Invitrogen) and PRIMER 3 internet-based primer

design utility (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), using the conditions described in King *et al.* (2001). A 19-bp fragment of the M13 universal primer was tagged to the 5′ end of each forward primer and a 4-bp M13 tag was added to the 5′ end of each reverse primer sequence (Boutin-Ganache *et al.* 2001).

Microsatellite DNA amplification reactions consisted of 100–200 ng of genomic DNA, 1× polymerase chain reaction (PCR) buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 2 mM $MgCl_2$, 0.25 mM dNTPs, 0.3 Units BSA (bovine serum albumin), 0.1 U *Taq* DNA polymerase (Promega) in a total volume of 10 μL, 0.3 μM forward, 0.6 μM reverse primer, and 0.12 μM fluorescent labelled primer, having the same universal sequence as the tagged forward primer. Amplifications were carried out on either a PTC-200 or PTC-225 thermal cycler (MJ Research) using this cycling regime: initial denaturing at 94 °C for 15 min; 29 cycles at 94 °C for 1 min; 58 °C for 45 s, 72 °C for 45 s; 5 cycles at 94 °C for 1 min; 53 °C for 45 s, 72 °C for 45 s and a final extension at 72 °C for 10 min. Fragment analysis and allelic designations followed techniques described in King *et al.* (2001).

Twenty-three primer pairs produced unambiguous PCR products interpreted as allelic variation. Locus designation, primer sequences, and repeat motifs for the *D. kirtlandii* markers, as well as, the range of PCR product sizes, number of alleles, and direct count and expected heterozygosities observed among the 14 overwintering warblers are provided in Table 1. Among the warblers sampled from Eleuthera, the observed genetic diversity ranged from three (*Dki*B3, *Dki*C118) to 10 (*Dki*D1, *Dki*D104, *Dki*D109) alleles and averaged to 7.7 alleles per locus. This level of diversity was sufficient to produce unique multilocus genotypes for each individual genotyped and resulted in a probability of 2.5×10^{-9} that two siblings in this *D. kirtlandii* sample, surveyed with the present suite of loci, have identical genotypes (PI_{sibs}; Taberlet & Luikart 1999).

Observed individual heterozygosity ranged from 21.4% (*Dki*C118) to 92.9% (*Dki*D1, *Dki*D104, *Dki*D123) and averaged 72.0% (SE 3.6%). Tests for conformance to Hardy–Weinberg equilibrium (GENEPOP version 3.2; Raymond & Rousset 1995) of the overwintering warblers indicated that one locus (*Dki*D117; heterozygote deficiency) deviated from expectations after the sequential Bonferroni correction for multiple simultaneous tests (Rice 1989) (overall $\alpha = 0.05$, $P < 0.002$). The global test for all 23 loci was not statistically significant ($P = 0.5399$). Among 251 possible pairwise locus comparisons, no linkage disequilibrium (GENEPOP) was detected.

We believe that these loci will yield sufficient genetic diversity to resolve patterns of effective migration among known *D. kirtlandii* colonies on the breeding grounds. Moreover, the availability of such a large suite of markers and the resulting multilocus genotypes should reduce the amount of resources required in initiating new genetic studies assessing breeding structure, parentage, demographics, and individual-level ecological interactions for *D. kirtlandii* and potentially, for other species in the family Emberizidae.

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